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On the Heat Production and Oxidation Processes of the Echinoderm Egg during Fertilisation and Early Development.

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Introduction.

In the following experiments an attempt is made to measure the heat liberation of the ovum on fertilisation and early development, and to correlate this with the amount of oxygen consumed and the carbon dioxide given off at the same time. New methods hitherto unused for this purpose have been employed. The question has already been investigated by Meyerhof (1) in an extensive paper published in 1911. He determined the heat production and the oxygen consumption of the egg of the sea-urchin *Strongylocentrotus* on fertilisation and early development. The heat production was measured directly by means of a finely divided Beckmann thermometer, while the eggs were contained in a small closed vacuum flask completely submerged in the water of a carefully regulated thermostat. The oxygen consumption of the eggs was at the same time determined at intervals of an hour, by the titration of the sea-water in which the eggs were kept with sodium thiosulphate by the Winkler method.

The heat given off by a known quantity of eggs expressed in gram calories per hour, divided by the amount of oxygen consumed in the same time expressed in milligrams, gave him a calorific quotient which he calls "Q." This he found for the early stages of segmentation to be about 2·75, but if the heat of solution of carbon dioxide to form bicarbonate with the sea-water is taken into consideration this value becomes 2·6. This figure is so low, however, as to suggest that his data for the heat liberation or the oxygen consumption are incorrect, or that the oxidation processes of the egg-cell on fertilisation are of a different character from those of adult metabolism. It has been shown by Zunst and Schumberg, Rauber, Pflüger and others, that when fat is consumed this figure should be in the vicinity of 3·3, when protein 3·2, and carbohydrate 2·9. Meyerhof could find no carbohydrate in the egg, and there could be no destruction of protein, but sufficient fat was found in the egg to give the quotient observed. In the case of fresh sperm, Q was 3·1 or nearly normal. The carbon dioxide production by the eggs was not measured.

The most important fact, however, arising from Meyerhof's experiments was that, whether he took the unfertilised egg, the fertilised, or the fertilised egg treated with phenylurethane, so that cell formation was inhibited although development proceeded, he found the value of this calorific quotient was always the same. If any of the chemical energy liberated in the egg as the result of the increased oxygen consumption of the egg on fertilization were utilised in producing the visible morphological structure of the egg, then the value of this quotient could not be the same in all these instances. Warburg (2) had already pointed out, that the oxygen consumption of the egg-cell on development always fails to keep pace with the increase in morphological structure. In *Arbacia* he found the fertilised egg in the one cell stage during the first hour of development consumed 4 c.c. of oxygen; in the sixth hour, the same quantity of eggs consumed only 6·8 c.c., although now the eggs were each composed of thirty-two cells instead of one.

In another experiment where a larger number of eggs were employed, 13·2 mgrm. of oxygen was consumed by the eggs in the eight-cell stage, while in the thirty-two cell stage only 20·5 mgrm. was absorbed. Thus, while the oxygen consumption doubled in amount the cellular structure had increased four-fold.

Meyerhof found the heat production of a quantity of unfertilised eggs containing 140 mgrm. of nitrogen (about 17 million eggs) to be about 0·9 grm. calories per hour, while the same quantity of fertilised eggs, liberated 4—4·2 grm. calories in this time. In the second hour, the two-cell stage, the heat production rose to 4·5–5 grm.-calories. In the fourth hour, corresponding to the 8-cell stage, it was 6–6·5 grm.-calories. In the sixth hour, the thirty-two-cell stage, it was 9·8 grm.-calories, and from this time onwards the heat liberation increased rapidly, until in the eighteenth hour, when the free swimming stage was reached, it was 17·8 grm.-calories per hour, or four times what it was in the first hour of development. Once development was initiated the heat production rose steadily without pause or interruption. It followed the oxygen consumption closely in all respects, and like this, showed no direct relationship to the rate at which morphological organisation took place within the egg. No heat production could be observed during the formation of the fertilisation membrane or the early phases of the fertilisation process itself.

In all Meyerhof's experiments great over-crowding of the eggs necessarily took place, and it is difficult to believe that under such conditions the heat production was normal. In attempting to repeat his experiments, using a much larger vacuum flask and a smaller quantity of sea-urchin eggs where they were less crowded, I was unable to get them to fertilise in the closed flasks. As Loeb first pointed out, an abundant oxygen supply is the invariable constant required by the fertilised and developing egg-cell. In my own experiments, in order to get my eggs to fertilise and segment regularly, I was forced to adopt some means of keeping them aërated during the course of the experiment. If large quantities of eggs were employed (300–400 mgrm. of egg nitrogen), then it was absolutely necessary to carry out artificial aëration, or otherwise a large number of the eggs quickly died and soon cytolysed, and during cytolysis liberated an abnormal amount of heat. As I have shown with bacteria (3), the death process and cytolysis of all cells is probably accompanied by an abnormally high oxygen consumption and heat liberation. On these grounds Meyerhof's experiments seemed open to criticism. It was worth while repeating his experiments, using different methods which avoided, as far as possible, this difficulty. Moreover, it was of interest to determine if a different method of measuring the heat liberation would give figures similar or of the same order as those obtained by Meyerhof.

The Winkler titration method employed by Meyerhof in estimating the oxygen consumption of the egg on fertilisation and development is somewhat unsatisfactory in that it probably gives too high a figure for the oxygen consumption of the egg. The sea-urchin egg on fertilisation discharges a certain amount of organic slimy material into the sea-water, which interferes to a considerable extent with the accuracy of the titrations carried out by this method. The following experiments are for these reasons, to a large extent, a repetition of Meyerhof's work, using different methods for both the heat measurement and the oxygen consumption and carbon dioxide output of the egg. The eggs and sperm of *Echinus miliaris* were employed. This species being a shore form it is exceptionally favourable for work of this kind. It can be readily reared to the adult stage in small culture jars under laboratory conditions. I have shown, working in conjunction with

De Morgan and Fuchs (4), that this species can be readily raised to the sexually mature F_2 generation in the laboratory if a few simple rules are followed in rearing the larvæ.

Methods.

In making the heat measurements the differential calorimetric method has been adopted instead of the direct method employed by Meyerhof. It requires no expensive fittings or elaborate thermostats, and has the advantage that a number of separate determinations can be made at the same time. All the following experiments were carried out so that the eggs were efficiently aërated. This was carried out so as not to interfere with the accuracy of the heat estimations. To test this point many preliminary experiments were made.* All final calibrations were carried out under conditions identical with those of an actual experiment; the mean of 30 or 40 determinations being taken as the final figure.

The oxygen and carbon dioxide determinations were carried out by the employment of a special pattern of the Barcroft (5) differential manometer, in which it was possible to fertilise the eggs in the closed chamber of the instrument. It was thus possible to record the oxygen consumption and carbon dioxide output of the eggs while the sperm were actually making their way into the egg. As this instrument and the mode of its use has already been described in a previous paper (6), it is unnecessary to give an account of it here.

In the heat measurements, the form of differential calorimetric method employed has been that devised by A. V. Hill (7), and has already been clearly described by him at some length. The method is based on the fact that, within fairly wide limits, a vacuum flask may be given any desired rate of conduction of heat to the outside by simply increasing or decreasing the volume of its fluid contents. By placing the right quantity of fluid, in this case eggs in sea-water, in one flask, and an appropriate quantity of plain sea-water in another flask acting as a control, the two flasks can be given the same temperature fall. They can then be used in making a differential determination, on being connected with one another by means of a thermocouple, with one junction in each flask. The thermocouple being in circuit with a delicate galvanometer, any deflection of the mirror gives the difference of temperature between the two flasks. A copper-constantan thermocouple was used in circuit with a sensitive Ayrton-Mather galvanometer. The sensitivity of the galvanometer was such that, at 3·5 metres distance, every millimetre of the galvanometer scale represented 0·00139° C. The leads from the galvanometer and thermocouples were brought to a specially constructed dial box furnished with two keys, by which three or four thermocouples could be thrown into circuit with the galvanometer, and also small resistances introduced in any of these circuits as desired. All leads and terminals, including those of the galvanometer, were made of copper throughout, thus avoiding any possible thermo-electric effects.

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* Aëration was carried out by bubbling a very small volume of water-saturated air simultaneously through both flasks at regular intervals, the eggs in the flask being previously well aërated for 20 minutes before the commencement of the experiment.

The vacuum flasks were the ordinary narrow-necked silvered Dewar flasks, made as "refills" for commercial thermos bottles. They were used in two sizes, having a capacity of 400 c.c. and 800 c.c. respectively. The larger size have a coefficient of heat loss half that of the smaller, and are therefore more accurate to work with where sufficient experimental material can be obtained. The selection of the flasks was carried out in the following manner:—Some 40 to 50 flasks were obtained for rough testing. These were all filled with the same quantity of water at 60° C. They were then closed with plugs of cotton wool, and put aside in a corner of the room free from draughts, and allowed to warm up for an hour. Their temperature was then taken with a Beckmann thermometer, after which they were allowed to stand for 24 hours, when their temperature was again taken with the Beckmann thermometer. It was usual to find four or five flasks out of the lot that had very similar rates of temperature fall, and these were selected for further calibration. Their coefficients of heat loss were then carefully worked out, under conditions as similar as possible to those obtaining in experiments by the use of the formula $T-T_0/A-T_0\,e-kt$, a mean of ten or twelve determinations being taken. The final calibration was carried out under actual conditions of an experiment, with air bubbling through the flask contents, and air tubes and thermocouple junctions in position, and the flask itself sunk down in the water of the thermostat. One flask was given a slightly higher temperature than the other, which was the exact temperature of the bath water; as the temperature of the flask under calibration fell slowly to that of the control flask, a series of readings were taken with the thermocouple and galvanometer; these, on being plotted out, gave a curve from which the value of k could be directly taken. The value given by this last method was the one actually employed for experiments. As a matter of fact, both methods gave very similar values for k in nearly all instances. Prof. Hill was kind enough to place at my service two flasks of 400 c.c. capacity, which were remarkable for having almost the same value of k. They could be used differentially by placing the same amount of fluid in each. They have been extensively used in the present experiments.

The flasks during the course of work were recalibrated from time to time, and at long intervals were tested by the liberation of a known amount of heat in each flask from a small coil of constantan wire. This coil liberated 21 grm.-calories of heat per hour in the flask under the conditions of the test, and the galvanometer scale readings were usually within 3 per cent. of this value. To close the mouths of the flasks during an experiment, it was found that thick wads of cotton wool were the most effective. When the flasks were closed by rubber stoppers and the flask sunk completely in the water of the thermostat, it was found that more heat was lost by conduction through the stopper than was the case when they were only plugged with cotton wool and sunk up to their necks in the water of the bath. The flasks were mounted in pairs in open wirework baskets, which were made so that they could be clamped on the thermostat, so the flasks were held firmly submerged up to within a centimetre of the tops of their necks in the water of the bath. The thermostat tank held 50 or 60 litres of water, and was kept stirred and in uniform temperature throughout, by having compressed air bubbled through it from a number of jets distributed evenly over the bottom of the tank. This method of stirring was very effective, for, when it was in action, it was seldom possible to distinguish more than a hundredth of a degree C. between any two points in the water of the tank. The sides of the tank were protected externally by thick layers of felt, and its inner side was surrounded by a coil of piping through which cold water could be circulated, and the temperature of the tank kept constantly at 14·5° C. The room in which the experiments were conducted was almost entirely underground, and underwent little change of temperature between day and night, or from one day to another, if the door was kept closed and the windows protected. The experiments were carried out in the months of July, August and September, when weather conditions were most favourable for work of this kind. It is the special merit of the differential method that external temperature conditions can be largely neglected, so long as both flasks used in making the differential determinations are affected to the same extent by all variations of external temperature.

In order to get the eggs to fertilise and segment regularly in the flasks, it was found necessary to carry out some form of aëration. To accomplish this, air was slowly bubbled through the contents of both flasks at regular intervals during an experiment. This also served to stir up the eggs and prevent their settling in a dense mass in the bottom of the flasks. The air used in the aëration and stirring process was first passed through a large wash-bottle, half-filled with sea-water, sunk in the middle of the thermostat tank. The air from this bottle was then led into each flask by fine rubber tubes, which passed through the cotton plugs used to close the flasks. The air thus saturated with moisture and at the same time brought to the temperature of the water bath, as Hill has shown, the heat capacity of air being so low, produces little or no cooling effect on the contents of the flasks. At the commencement of an experiment care was taken to adjust both flasks, but especially the control flask, to exactly the same temperature as the thermostat water, which, as already mentioned, was kept constantly at 14·5° C. This adjustment in the case of the control flask was always made to within a hundredth of a degree C. with a Beckmann thermometer. This adjustment was usually carried out several times in succession before an experiment was actually commenced. The sperm were suspended in a small bottle in the water of the thermostat, so that when finally added to the eggs in the flask they were at approximately the same temperature.

On the addition of the sperm the cotton plugs, with the thermocouple junctions and air tubes, were immediately replaced in the necks of the flasks, and galvanometer readings commenced. Readings were always taken at fairly frequent intervals at the commencement of an experiment, but once the experiment was under way, they were usually taken at intervals of several hours. The readings obtained, in millimetres on the galvanometer scale, were then plotted out on squared paper with respect to time, and a curve of observed heat production obtained. As the flasks are meanwhile losing heat, a correction for heat loss has to be made. The loss of one flask, however, by the conditions of the experiment has been made the same as that of the other, so that the rate of temperature-rise in the flask containing the eggs is immediately given by the formula $k(T-T_1)$, where k is the coefficient of temperature-loss of the flask, and $(T-T_1)$ is the difference of temperature between the two flasks, as shown by galvanometer scale readings in millimetres at any instant. The total temperature-rise in the flask is obtained by integrating $k(T-T_1)$ with respect to time, and this value is accurately given by measuring the area of the curve given by the galvanometer scale readings plotted against time. The total heat produced is equal to the capacity of the flasks and fluid multiplied by the final temperature difference between the flasks plus this value of k (times area of curve), where this last expression is equivalent to k [value of the middle ordinate of $(T-T_1)$].

In the following sections of the paper, a few only of the many experiments carried out have been described. In many instances the eggs or sperm, for one reason or another, were unsatisfactory and the experiment failed to give a result. In other experiments, while the eggs and sperm were perfectly ripe and the eggs gave a very high percentage of fertilisation, they failed to fertilise in the manometers to the same extent as they did in the flasks, so rendering comparison of the oxygen intake with the heat production impossible. It is essential in experiments of this kind that in both the oxygen and CO_2, and also the heat determinations, all the eggs should fertilise at the same time, and that, after fertilisation, they should all develop at the same rate, as otherwise no comparisons can be made between different portions of the experiment. In all, out of some 500 experiments few were satisfactory in all respects.

To simplify matters the manometer readings in the following experiments have all been reduced to standard barometric (760 mm. Hg.) pressure and uniform temperature of 14·5° C. The galvanometer scale readings have also been adjusted to start from zero, although it was seldom possible to adjust the temperature of the two flasks so closely that the readings should actually commence at zero. The galvanometer mirror deflection being always either to the right or left the zero was in the middle of the screen. The thermocouple was arranged so that the hottest junction should always deflect the mirror to the right.

Experiment 1.

(a) *Heat Determination.*—400 c.c. of ripe well washed *E. miliaris* eggs in sea-water were placed in Flask R, and 380 c.c. sea-water in Flask No. 7, under the conditions of the experiment both flasks had the same coefficient of heat loss. The flasks were sunk down in the bath water, with the thermocouple junctions and air tubes in place. The temperature of the flasks was adjusted to within a hundredth of a degree of the temperature of the bath, and the flasks allowed to remain with air bubbling through them for half-an-hour. The flasks were closed with thick wads of cotton-wool. At the end of this time the temperature of the flasks was again adjusted as near as possible to that of the bath, and after a few minutes a few drops of sperm were added to the eggs, and the cotton plugs with air tubes and thermocouple junctions replaced in the flasks and galvanometer readings commenced.

Time 3 P.M. galvanometer scale reading was 0
„ 4 „ „ „ 5 mm. to R containing eggs.
„ 5 „ „ „ 7 „ „ „ „
„ 6 „ „ „ 10 „ „ „ „
„ 11 „ „ „ 21 „ „ „ „
„ 2 A.M.* „ „ 28 „ „ „ „

At the end of the experiment 98–100 per cent. of the eggs in the flask were in normal morula stages. Kjeldahl determination gave 58·4 mgrm. egg nitrogen present. In the first hour after the addition of the sperm the eggs liberated 2·9 grm.-calories, 10·5 grm.-calories in the fifth hour, and 22·8 grm.-calories in 11 hours (see Curve, fig. 1, *a*).

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* In carrying out an experiment the preliminary preparations often took up so much time that the actual galvanometer readings could only be started late in the day and had sometimes to be carried through to the following morning.

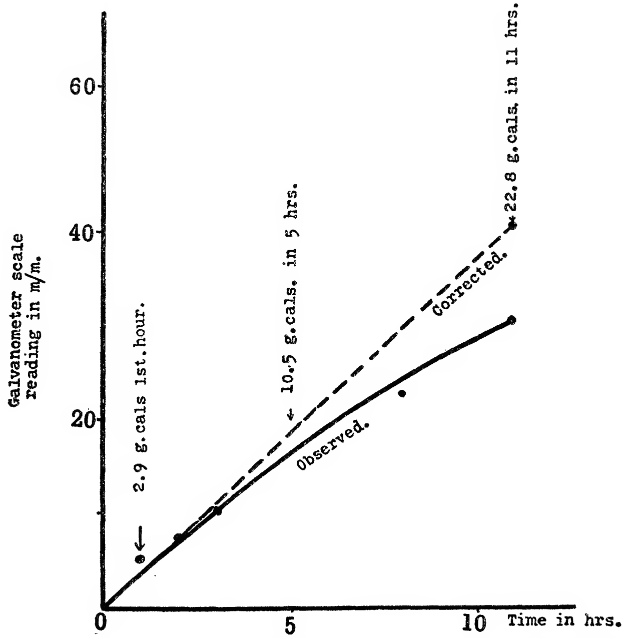

Fig. 1, *a*.—Curve of heat production for Experiment I. 58·4 mgrm. egg nitrogen.

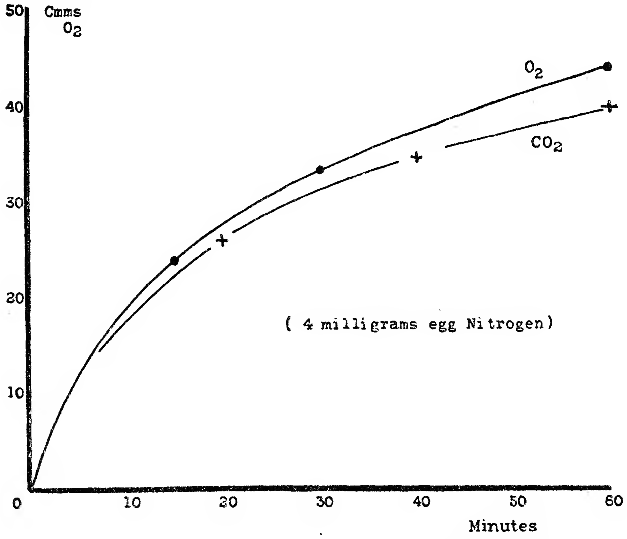

Fig. 1, *b*.—Curve of O_2 consumption and CO_2 liberation for same Experiment I. 4·07 mgrm. egg nitrogen.

(b) *Oxygen and Carbon Dioxide Determination.*—2 c.c. of the same lot of eggs as those placed in the flask in above experiment were put in the chamber of oxygen manometer and a drop of KOH put in the cup, 2 c.c. of plain sea-water being placed in the control chamber. In a second manometer used for control the same quantity of eggs were placed in the chamber, the KOH being left out.

Temperature of manometer bath 14·5° C., barometer 760 mm. The manometers were put in position in the bath and after being brought into complete equilibrium with the bath water the cocks of the manometers closed and the eggs fertilized. The first manometer showed that:—

At the end of 15 minutes the eggs had consumed 24·2 c.mm. oxygen.
" " 30 " " " 33·0 " "
" " 60 " " " 44·0 " "

At the end of the experiment eggs in the chamber showed 100 per cent. fertilisation membranes and some commencing two cell stages. Kjeldahl determination on the eggs gave 4·07 mgrm. egg nitrogen present. 58·4 mgrm. of egg nitrogen at this rate would take up 631·4 c.mm. oxygen, which is equivalent to 0·902 mgrm.

As 2·9 grm.-calories of heat were given off in the first hour following fertilisation, for 58·4 mgrm. N in first part of experiment,

$$Q = \frac{2{\cdot}9}{0{\cdot}902} = 3{\cdot}215.$$

The heat production in this experiment was 10·5 grm.-calories in 5 hours; in 12 hours, 22·8 grm.-calories.

In the second manometer 4·0 c.mm. of oxygen seemed to be the difference between the oxygen intake and CO_2 output in this experiment, for 4·07 mgrm. of egg nitrogen. If we assume that 44·0 − 4·0 gives us the amount of carbon dioxide produced in this experiment, we get the respiratory quotient of $40/44 = 0{\cdot}91$.

It will thus be seen the carbon dioxide output of the eggs is almost as great as the oxygen consumption. In all the experiments the carbon dioxide respiration follows the oxygen consumption very closely, the respiratory quotient varying from 0·9 to 0·95 in different experiments.

Experiment 2.

(a) *Heat Determination.*—800 c.c. of well washed ripe *E. miliaris* eggs in sea-water placed in Flask No. 3, 763 c.c. sea-water placed in Flask No. 4 acting as a control. Under the conditions of the experiments with air bubbling through both flasks and thermocouple junctions in position, and the flasks sunk in the water of the thermostat, k for the first flask was 0·0238, and for the second $k = 0·0236$. Temperature adjusted all round to within a hundredth of a degree to 14·5° C. Cooled sperm added and readings started.

Time 3.30 P.M.	galvanometer scale reading	0
„ 6.30 „	„ „	17 mm. R flask containing eggs.
„ 9.30 „	„ „	26 „ „ „ „
„ 12.30 A.M.	„ „	47 „ „ „ „
„ 2.30 „	„ „	59 „ „ „ „

At end of experiment 95–100 per cent. of eggs were in healthy free-swimming early gastrula stages. Kjeldahl determination gave 146·2 mgrm. of egg nitrogen present in flask contents. The heat liberated in first hour 6·34 grm.-calories, in the fifth hour 28 grm.-calories, and in the eleventh hour 74·4 grm.-calories (see Curve, fig. 2, *a*).

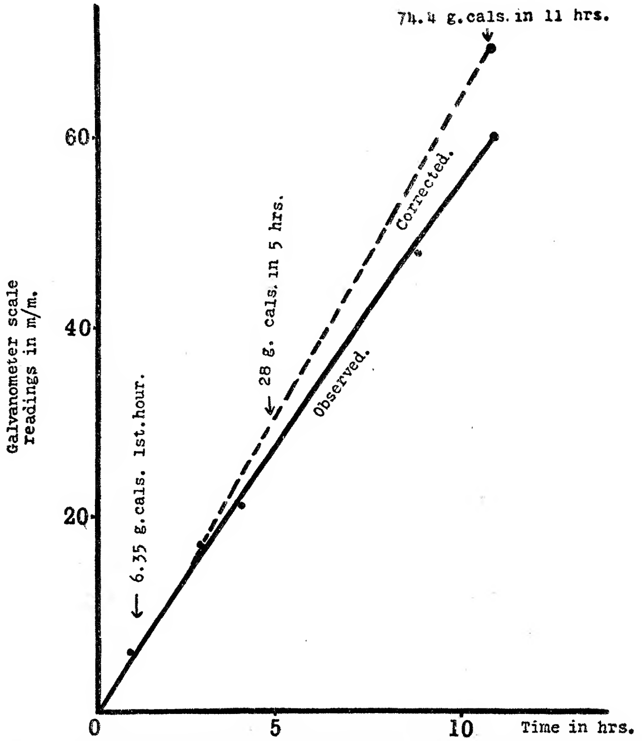

FIG. 2, *a*.—Curve of heat production to Experiment II. 146·2 mgrm. egg nitrogen.

(b) *Oxygen and CO_2 Determination.*—2 c.c. of same lot of egg material as that put in the flask in the first part of the experiment, placed in a chamber

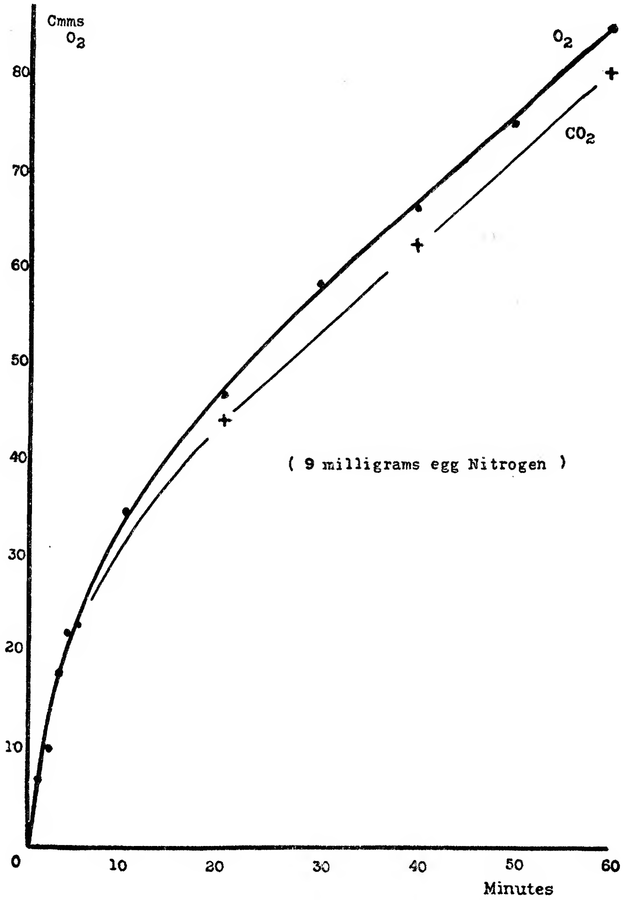

FIG. 2, *b*.—Curve of O_2 consumption and CO_2 liberation for Experiment II. 9 mgrm. egg nitrogen.

of oxygen manometer, and the same quantity in a chamber of CO_2 apparatus. The eggs were fertilised. Those in the oxygen manometer showed that at the end of

1 minute after addition of the sperm eggs had consumed 6·95 c.mm. oxygen.
2 minutes „ „ „ „ 10·0 „ „
3 „ „ „ „ „ 18·0 „ „
4 „ „ „ „ „ 22·0 „ „
5 „ „ „ „ „ 23·05 „ „
10 „ „ „ „ „ 34·5 „ „
20 „ „ „ „ „ 47·0 „ „
30 „ „ „ „ „ 58·3 „ „
40 „ „ „ „ „ 66·2 „ „
60 „ „ „ „ „ 85·0 „ „

At end of experiment 100 per cent. of eggs showed fertilisation membranes and commencing two-cell stage. Kjeldahl determination gave 9 mgrm. egg nitrogen present.

At this rate 146·2 mgrm. egg nitrogen would consume 1380 c.mm. oxygen in the first hour following fertilisation, 1380 c.mm. oxygen being equivalent to 1·97 mgrm.

The heat produced by 280 mgrm. egg nitrogen in the first hour following fertilisation, as shown in the early part of this experiment, was 6·35 grm.-calories. So that

$$Q = \frac{6{\cdot}35}{1{\cdot}97} = 3{\cdot}22.$$

The heat production rose in the fifth hour to 28 grm.-calories, and to 74·4 grm.-calories in the 11 hours in this experiment. The corresponding CO_2 determination for this experiment gave a respiratory quotient of 0·95.

Experiment 3.

800 c.c. of ripe well washed *E. miliaris* eggs were placed in Flask No. 3, 763 c.c. plain sea-water being placed in Flask No. 4, acting as a control under the conditions of the experiment with flasks sunk in the water of the thermostat and air-bubbled through both flasks, k for flask No. 3 was 0·0238 while that for Flask No. 4 was 0·0236. Temperature was adjusted all round to within a hundredth of a degree to 14·5° C. No sperm were added. Galvanometer readings were commenced. At the end of 1 hour Flask No. 3 had given off 3·6 grm.-calories of heat. A Kjeldahl determination on the flask contents gave 431 mgrm. egg nitrogen present. In the same time 8 mgrm. egg nitrogen of the same batch of egg material, consumed 15·1 c.mm. oxygen. Therefore 431 mgrm. of egg nitrogen would consume at this rate 812 c.mm. oxygen in this time, as 812 c.mm. oxygen equal 1·17 mgrm. O_2 we get value of

$$Q = \frac{3{\cdot}6}{1{\cdot}17} = 3{\cdot}07$$

for the unfertilised egg. The value obtained for the fertilised egg of *E. miliaris* in the two previous experiments was 3·215 and 3·22 respectively. Thus the value of Q is somewhat different in the two cases.

It is doubtful, however, if much significance can be attached to this difference. The gonads of so many females have to be used for making determination on the heat production of the unfertilised egg, that it is impossible that they should all be in the same stage of ripeness. Some of the gonads are certain to be slightly over ripe, and their eggs will probably cytolyse on being placed in the vacuum flask, and will give off an abnormally large amount of heat; others, again, will be somewhat immature, and will consequently give off little heat as compared with the properly mature stage. On the whole, from a number of experiments with the unfertilised egg, I am inclined to think there is little difference between the value of Q in the unfertilised, as compared with the fertilised egg, and that Meyerhof's conclusion that it is the same for both is correct. In three successful experiments with the unfertilised egg, the figure for Q obtained in the above described experiment represents the mean, and it is worth noting that this value is slightly smaller than in the case of the fertilised egg.

Discussion.

The Unfertilised Egg.—In the foregoing experiments it has been shown that the oxygen consumption and the heat liberation of the unfertilised egg of *E. miliaris* is remarkably small. In 1 hour, 1,000,000 eggs (8 mgrm. egg nitrogen) only consumed 15·1 c.mm. of oxygen, and liberated at the same time something of the order of 0·067 grm.-calorie heat. If we divide the heat liberation in 1 hour's time, expressed in gramme calories, by the oxygen consumption in milligrams, we get a quotient which we may call the calorific quotient. In the case of the unfertilised egg this quotient was found to be 3·07. Meyerhof, in *Strongylocentrotus,* using the Winkler method for estimating the oxygen consumption and the direct method for measuring the heat production, found this egg consumed 9·41 c.mm. of oxygen and liberated 0·038 grm.-calorie, under similar conditions. The value of Meyerhof's calorific quotient (Q) varied about 2·8.

The Fertilised Egg.—On the addition of the sperm to the eggs of *E. miliaris,* there is an immediate oxygen consumption by the egg and a corresponding increase in the heat liberated. At the end of the first hour of development 86·4 c.mm. of oxygen were consumed by 1,000,000 eggs (8 mgrm. egg N.), and 0·397 grm.-calorie of heat was liberated. The CO_2 output of eggs was almost the same as the oxygen intake; the respiratory quotient being in the vicinity of 0·92. In *Strongylocentrotus,* Meyerhof found under similar conditions for 65·38 c.mm. of oxygen consumed a heat liberation of 0·247 grm.-calorie, for a similar quantity of eggs. The calorific quotient in this instance again being 2·6 to 2·8. In view of the large number of eggs that have to be used for making a heat determination on the unfertilised egg, it is doubtful if much significance attaches to the difference found between the calorific quotient of 3·07 in the unfertilised egg, as compared with 3·2 in the fertilised condition.

Summary.

1. In the present paper an attempt has been made to measure the oxygen consumption of the egg of *Echinus miliaris* on fertilisation and early development and compare it with the amount of heat liberated by the egg at the same time.

2. In making both these estimations new methods have been employed.

3. The oxygen consumption of the egg has been measured by the use of a special pattern of the Barcroft differential manometer, in which the eggs were fertilised within the closed chambers of the apparatus. The CO_2 output of the egg was also measured with the same instrument and the respiratory quotient determined.

4. The heat liberation was measured by the use of the differential micro-calorimetric method.

5. In 1 hour 1 million unfertilised eggs (8 mgrm. egg N.) consumed 15·1 c.mm. of oxygen and gave off at the same time 0·067 of a gram-calorie of heat at standard pressure (760 mm. Hg.) and temperature 14·5° C.

6. In the same time the same quantity of fertilised eggs consumed 86·4 c.mm. oxygen (with a corresponding output of CO_2, respiratory quotient 0·92) and liberated 0·3976 of a gram-calorie of heat under similar conditions.

7. The fertilised egg in the first hour of development gave off roughly 6—7 times more heat than the unfertilised egg and consumed at the same time 6 or 7 times more oxygen than the unfertilised egg.

8. In the fertilised egg in one experiment (I) 58·4 mgrm. egg nitrogen (about 7·3 million eggs) liberated 2·9 gram-calories at the end of the first hour of development; in the fifth hour, 10·5 gram-calories and 22·8 gram-calories of heat in 11 hours. In another experiment (II) 146·2 mgrm. egg nitrogen (18·6 million eggs) liberated 6·35 gram-calories in the first hour, 28 gram-calories in the fifth hour, and in 11 hours 74·4 gram-calories. (This last figure is possibly too high, due to some cytolysis.) On the whole the heat liberation of the egg on fertilisation rises steadily, reaching its highest point when segmentation has been completed and the free-swimming stage is reached.

9. The heat liberation of the egg during the first hour after the sperm have been added to the eggs expressed in gram-calories divided by the amount of oxygen consumed in the same time, expressed in milligrammes, gives a calorific quotient (Q).

10. In the case of the unfertilised egg the calorific quotient was found to be about 3·07, while in the fertilised egg it was found to be 3·22.

11. On fertilisation a greatly increased liberation of chemical energy is brought about within the ovum. This is shown by the increased oxygen consumption of the fertilised egg-cell combined with its greatly increased carbon dioxide and heat liberation.

12. As, however, the calorific quotient of the unfertilised and the fertilised egg-cell is approximately the same in both instances, little or almost a negligible quantity of this energy is expended in bringing about the visible morphological structure of the developing ovum. It is probably employed in keeping the living substance itself intact as a physical system.

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